

PATENT COOPERATION TREATY

PCT


INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

REC'D 19 OCT 2005

PCT

Applicant's or agent's file reference 089548-0129		FOR FURTHER ACTION		See Form PCT/PEA/416
International application No. PCT/US2004/007897		International filing date (day/month/year) 16.03.2004	Priority date (day/month/year) 17.04.2003	
International Patent Classification (IPC) or national classification and IPC C12Q1/42, C07F9/09				
Applicant AMERICAN RED CROSS et al.				
<p>1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 10 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p>a. <input checked="" type="checkbox"/> sent to the applicant and to the International Bureau a total of 11 sheets, as follows:</p> <p><input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p>b. <input type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>				
<p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the opinion</p> <p><input checked="" type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input checked="" type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input checked="" type="checkbox"/> Box No. VI Certain documents cited</p> <p><input checked="" type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input type="checkbox"/> Box No. VIII Certain observations on the international application</p>				
Date of submission of the demand 17.02.2005		Date of completion of this report 18.10.2005		
Name and mailing address of the International preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 gpmu d Fax: +49 89 2399 - 4465		Authorized Officer Hennard, C Telephone No. +49 89 2399-7355		



**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/US2004/007897

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:

- ☐ international search (under Rules 12.3 and 23.1(b))
- ☐ publication of the international application (under Rule 12.4)
- ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-25 as originally filed

Sequence listings part of the description, Pages

1 as originally filed

Claims, Numbers

1-42 filed with telefax on 22.09.2005

Drawings, Sheets

1/8-8/8 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/figs
- ☐ the sequence listing (*specify*):
- ☐ any table(s) related to sequence listing (*specify*):

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/figs
- ☐ the sequence listing (*specify*):
- ☐ any table(s) related to sequence listing (*specify*):

* If item 4 applies, some or all of these sheets may be marked "superseded."

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Box No. II Priority

1. ☒ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
☒ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
☐ restricted the claims.
☒ paid additional fees.
☐ paid additional fees under protest.
☐ neither restricted nor paid additional fees.
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is
☐ complied with.
☐ not complied with for the following reasons:
4. Consequently, this report has been established in respect of the following parts of the international application:
☒ all parts.
☐ the parts relating to claims Nos. .

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-42
	No: Claims	None
Inventive step (IS)	Yes: Claims	1-42
	No: Claims	None
Industrial applicability (IA)	Yes: Claims	1-42
	No: Claims	None

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

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Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:
 - a. type of material:
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing:
 - ☒ contained in the international application as filed
 - ☒ filed together with the international application in computer readable form
 - ☐ furnished subsequently to this Authority for the purposes of search and/or examination
 - ☐ received by this Authority as an amendment on
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional observations, if necessary:

Re Item IV

Lack of unity of invention

The present claim were found to lack unity at the search stage. The inventions are grouped as follows:

- Claims 1-29: Compound of formula (I) and method for detecting or measuring the presence of organophosphatase enzyme in a fluid or immobilized on a solid support.
- Claims 30-42: Compound of formula (II) and method for detecting or measuring the presence of organophosphatase enzyme in a fluid or immobilized on a solid support.

The only concept which could possibly link the subject-matter of **claims 1-42** of the present application, as required by **Rule 13.1 PCT**, could be seen in providing a phosphodiester compound further characterised by having a bicyclic heteroaromatic moiety (chromene) attached to the phosphate, and which after hydrolysis by a phosphatase generates a fluorescent entity.

This concept is however known from the prior art (see Horne et al WO02/092803, pages 33-35 and figure 1) which describes coroxon and dMUP (which both fall within the scope of independent claim 1), among others, as substrates of organophosphate dihydrolase (a specific phosphatase). Further, Schabert et al (EP0949266; page 2, paragraphs 0006-0009; page 4, compound (II); page 9, example 3) describes MeU-phos-inositol which upon interaction with PI-PCL becomes fluorescent.

From these documents it is concluded that the concept linking the two above defined inventions is not new and cannot be seen as a common inventive concept. Therefore, the problem to be solved by the present application can be seen in the concept of providing new fluorogenic compounds bearing a phosphodiester in order to detect the presence of a phosphatase.

The compounds of formula (I) and (II) constitute therefore two different alternatives to the problem to be solved and the application as filed is considered to lack unity (rule 13.1 PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

**INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(SEPARATE SHEET)**

International application No.

PCT/US2004/007897

1. Reference is made to the following documents:

- D1: WO 02/092803 A
- D2: EP-A-0 949 266
- D3: BIOSCIENCE REPORTS, vol. 19, no. 2, April 1999, pages 81-87,
- D4: WO 03/020984 A
- D5: WO 03/020734 A
- D6: US-A-5 011 964
- D7: JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 268, no. 9, 1993, pages 6316-6322,
- D8: GB 972 981 A
- D9: US-A-3 457 332
- D10: US-A-5 998 593
- D11: US-A-5 981 207
- D12: ANALYTICAL BIOCHEMISTRY, vol. 273, no. 1, 1999, pages 41-48,
- D13: WO 03/088990 A
- D14: JOURNAL OF BIOMOLECULAR SCREENING, vol. 4, no. 6, December 1999, pages 327-334,
- D15: BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, vol. 9, no. 10, 17 May 1999, pages 1395-1396,
- D16: US-A-5 830 666
- D17: US-A-5 773 236
- D18: BIOCONJUGATE CHEMISTRY, vol. 12, no. 2, March 2001, pages 307-313,
- D19: BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1601, no. 1, 19 November 2002, pages 19-28

D13 which is an intermediate document, filed on 14.04.2003, published on 30.10.2003 and claiming a priority right on 15.04.2002, is not prior art according to the Chap II PCT proceedings (and will not be used at this stage (**Rule 70.10 PCT**)). Nevertheless, the Applicant is informed that the content of this document seems to affect the novelty of the present application and could thus become relevant in the national/regional phase. Moreover, should the priority of the present application not be valid, **D13** could become also relevant for inventive step.

A Preliminary opinion relating to the first invention represented by claims 1-29 and defined as follows:

Compound of formula (I) and method for detecting or measuring the presence

of organophosphatase enzyme in a fluid or immobilized on a solid support.

2. Novelty (Article 33(2) PCT):

By restricting the claims, the documents cited in the search report no longer affect the novelty of the present **claims 1-29**.

3. Inventive merit (Article 33(3) PCT):

D1(pages 3-9; pages 33 and 34), which is the closest prior art, discloses a method for detecting organophosphatases in a sample and provides different compounds to be used in this method.

The compounds of present **claim 1** distinguishes themselves from **D1** in that the acid functions of the phosphate are esterified by an ethyl group.

The technical effect obtained by these ester groups is that the compounds are suitable for the detection of paraoxonase.

Thus, the problem to be solved by the present **claim 1** is to provide a compound for **specifically** and **selectively** detect the organophosphatase (paraoxonase) activity in a biological fluid.

As demonstrated in example 8 of the description, compounds which are not phospho-esters are not suitable for the detection of paraoxonase. Therefore, the solution which consists in the use of compounds having the phosphate group esterified is not obvious in the light of **D1** and an inventive merit for these compounds can be recognised.

Consequently, the compounds of **claims 1-8** as well as the methods involving them (**claims 9-29**) are considered to demonstrate an inventive merit and fulfil the requirements of **Article 33(3) PCT**.

4. Industrial applicability (Article 33(4) PCT):

Due to the nature of the claims, an industrial applicability of the invention is obvious and **claims 1-29** of the present application are considered to fulfil the requirements of **Article 33(4) PCT**.

B Preliminary opinion relating to the second invention represented by claims 30-42 and defined as follows:

Compound of formula (II) and method for detecting or measuring the presence of organophosphatase enzyme in a fluid or immobilized on a solid support.

5. Novelty (Article 33(2) PCT):

Since none of the cited documents describes a fluorescein derivative bearing one or two substituted phosphate residues, the compound of **claims 30-39** and the methods of **claims 40-42** are considered to be novel and fulfil the requirements of **Article 33(2) PCT**.

6. Inventive merit (Article 33(3) PCT):

D15 (page 1395, scheme; page 1396, table and figure), which is considered to be the closest prior art, concerns fluorescein substrates for phosphatase enzyme in order to assay the enzyme.

The derivatives of **claim 30** of the present invention distinguish themselves from **D15** by the presence of a substituent different from hydrogen on the phosphates.

The technical effect achieved by these esters is in the specific detection of organophosphatases in a fluid, therefore, the problem to be solved by the present invention consists in providing new compounds suitable for the specific detection of organophosphatase.

Since phosphate substituted fluorescein analogues are not known from the prior art as substrates for organophosphatase, an inventive merit for the compounds of **claim 30** can be recognised. Thus, the compounds of **claims 30-39** and the method using them (**claims 40-42**) are considered to involve an inventive merit and fulfil the requirements of **Article 33(3) PCT**.

7. Industrial applicability (Article 33(4) PCT):

An industrial applicability of the invention is obvious and **claims 30-42** of the present invention are considered to fulfil the requirements of **Article 33(4) PCT**.

8. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in D1-D12 and D14-19 are not mentioned in the description, nor are these documents identified therein.

Re Item VI

Certain documents cited

Certain published documents

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
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**INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(SEPARATE SHEET)**

International application No.

PCT/US2004/007897

WO03/088990

30.10.2003

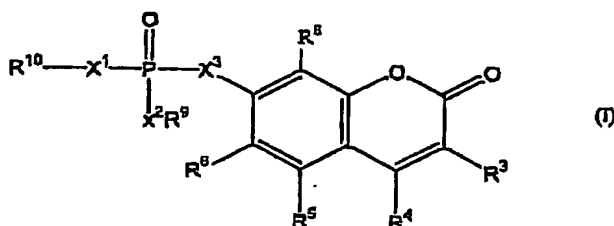
14.04.2003

15.04.2002

PCT/US2004/007897

WHAT IS CLAIMED IS:

1. A compound of the formula I:



wherein

R^3 is selected from the group consisting of H, cyano, C_1 - C_6 alkyl, C_1 - C_6 perfluoroalkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, aryl, and heteroaryl, formyl, carboxamide of the formula $-(C=O)NR^1R^2$ where R^1 and R^2 are independently H, alkyl having 1-6 carbon atoms, an aryl, or R^1 and R^2 taken together form a saturated 5- or 6- membered ring having the formula $-(CH_2)_2-M-(CH_2)_2-$ where the ring moiety M is a single bond, an oxygen atom, a methylene group, or the secondary amine $-NR^7-$ where R^7 is H or alkyl having 1-6 carbon atoms;

R^4 is selected from the group consisting of H, hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, sulfomethyl, salt of sulfomethyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, guanidino, C_1 - C_6 alkylamino, C_1 - C_6 acylamino, C_1 - C_6 alkylamido, C_1 - C_6 alkyl, C_1 - C_6 alkoxy, C_1 - C_6 perfluoroalkyl, halomethyl, C_1 - C_6 alkylthio, C_5 - C_8 cycloalkyl, C_1 - C_6 haloalkyl, C_5 - C_8 halocycloalkyl, C_1 - C_6 hydroxyalkyl, C_5 - C_8 hydroxycycloalkyl, C_1 - C_6 alkoxy C_1 - C_6 alkyl, C_2 - C_6 alkoxycarbonyl, C_2 - C_6 alkoxycarbonyl C_1 - C_6 alkyl, carboxy C_1 - C_6 alkyl, carboxy C_1 - C_6 alkoxy, dicarboxy C_1 - C_6 alkyl, dicarboxy C_1 - C_6 alkoxy, C_2 - C_6 cyanoalkyl, phosphono C_1 - C_6 alkyl, phosphoryl C_1 - C_6 alkyl, mono-, di-, and trisaccharides, nucleic acids, oligonucleotides, amino acids, peptides, and proteins, and C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, aryl, arylcarbonyl, and heteroaryl, which may be optionally substituted with a substituent selected from the group consisting of hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, and guanidine;

R^5 is H or C_1 - C_6 alkoxy;

R^9 and R^{10} are ethyl;

R^6 and R^8 are halo; and

X^1 , X^2 , and X^3 are independently O or S.

2. The compound of claim 1, wherein R^4 is selected from the group consisting of H, cyano, sulfomethyl, salt of sulfomethyl, aryl, C_1 - C_6 alkyl, C_1 - C_6 alkoxy, and C_1 - C_6 perfluoroalkyl.

3. The compound of claim 2, wherein R^4 is selected from the group consisting of C_1 - C_6 alkyl.

4. The compound of claim 3, wherein R^4 is methyl.

5. The compound of claim 1, wherein R^6 and R^8 are fluoro.

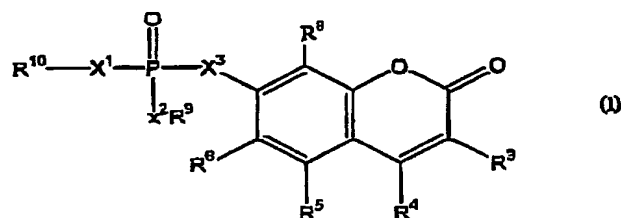
6. The compound of claim 1, wherein R^9 and R^{10} are ethyl, R^4 is methyl, and R^6 and R^8 are fluoro.

7. The compound of claim 1, wherein X^1 , X^2 , and X^3 are O.

8. The compound of claim 1, wherein X^1 , X^2 , and X^3 are S.

9. A method for specifically and selectively detecting and/or measuring the activity of an organophosphatase enzyme in a biological fluid, which contains at least organophosphatases and phosphatases, said method comprising:

(a) contacting the fluid with a compound of the formula I:



wherein

R^3 is selected from the group consisting of H, cyano, C_1 - C_6 alkyl, C_1 - C_6 perfluoroalkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, aryl, and heteroaryl, formyl, carboxamide of the formula $-(C=O)NR^1R^2$ where R^1 and R^2 are independently H, alkyl having 1-6 carbon atoms, an aryl, or R^1 and R^2 taken together form a saturated 5- or 6- membered ring having the formula $-(CH_2)_2-M-(CH_2)_2-$ where the ring moiety M is a single bond, an oxygen atom, a methylene group, or the secondary amine $-NR^7-$ where R^7 is H or alkyl having 1-6 carbon atoms;

R^4 is selected from the group consisting of H, hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, sulfomethyl, salt of sulfomethyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, guanidino, C_1 - C_6 alkylamino, C_1 - C_6 acylamino, C_1 - C_6 alkylamido, C_1 - C_6 alkyl, C_1 - C_6 alkoxy, C_1 - C_6 perfluoroalkyl, halomethyl, C_1 - C_6 alkylthio, C_5 - C_8 cycloalkyl, C_1 - C_6 haloalkyl, C_5 - C_8 halocycloalkyl, C_1 - C_6 hydroxyalkyl, C_5 - C_8 hydroxycycloalkyl, C_1 - C_6 alkoxy C_1 - C_6 alkyl, C_2 - C_6 alkoxycarbonyl, C_2 - C_6 alkoxycarbonyl C_1 - C_6 alkyl, carboxy C_1 - C_6 alkyl, carboxy C_1 - C_6 alkoxy, dicarboxy C_1 - C_6 alkyl, dicarboxy C_1 - C_6 alkoxy, C_2 - C_6 cyanoalkyl, phosphono C_1 - C_6 alkyl, phosphoryl C_1 - C_6 alkyl, mono-, di-, and trisaccharides, nucleic acids, oligonucleotides, amino acids, peptides, and proteins, and C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, aryl, arylcarbonyl, and heteroaryl, which may be optionally substituted with a substituent selected from the group consisting of hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, and guanidine;

R^5 is H or C_1 - C_6 alkoxy;

R^9 and R^{10} are ethyl;

R^6 and R^8 are halo or hydrogen; and

X^1 , X^2 , and X^3 are independently O or S;

(b) measuring the fluorescence of a fluorescent product formed during the contacting; and

(c) correlating the measured fluorescence with the activity of the organophosphatase enzyme.

10. The method of claim 9, wherein the organophosphatase is paraoxonase.

11. The method of claim 9, wherein the organophosphatase is OPH.

12. The method of claim 9, wherein R^9 and R^{10} are ethyl, R^4 is methyl, R^6 and R^8 are fluoro, and X^1 , X^2 , and X^3 are O.

13. The method of claim 9, wherein X^1 and X^2 are O, X^3 is S, R^6 and R^8 are H; R^9 and R^{10} are ethyl, and R^4 is methyl.

14. The method of claim 9, wherein the fluid is a biological fluid.

15. The method of claim 14, wherein the biological fluid is selected from the group consisting of blood, blood-derived compositions, serum, cerebrospinal fluid, urine,

saliva, milk, ductal fluid, tears, semen, cell or tissue expression of paraoxonase or mutations of paraoxonase or fractionation of paraoxonase or HDL from biological

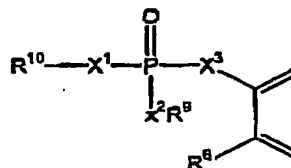
16. The method of claim 15, wherein the sample is from a vein or gland.

17. The method of claim 14, wherein the sample is from a

18. The method of claim 17, wherein the sample is from water, or swab.

19. A method for selectively detecting organophosphatase activity suspected to contain an organophosphatase and

(a) contacting the sample with a compound



wherein

R³ is selected from the group consisting of perfluoroalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, aryl, or formula -(C=O)NR¹R² where R¹ and R² are independently an aryl, or R¹ and R² taken together form a saturated ring moiety, formula -(CH₂)₂-M-(CH₂)₂- where the ring moiety is a methylene group, or the secondary amine -NR⁷, or atoms;

R⁴ is selected from the group consisting of amido, azido, acetal, ketal, imido, sulfo, sulfonyl, thiocyanato, aldehydo, keto, carbamoyl, urethan, C₆ acylamino, C₁-C₆ alkylamido, C₁-C₆ alkyl, C₁-C₆ halomethyl, C₁-C₆ alkylthio, C₅-C₈ cycloalkyl, (

hydroxyalkyl, C₃-C₆ hydroxycycloalkyl, C₁-C₆ alkoxy C₁-C₆ alkyl, C₂-C₆ alkoxy carbonyl, C₂-C₆ alkoxy carbonyl C₁-C₆ alkyl, carboxy C₁-C₆ alkyl, carboxy C₁-C₆ alkoxy, dicarboxy C₁-C₆ alkyl, dicarboxy C₁-C₆ alkoxy, C₂-C₆ cyanoalkyl, phosphono C₁-C₆ alkyl, phosphoryl C₁-C₆ alkyl, mono-, di-, and trisaccharides, nucleic acids, oligonucleotides, amino acids, peptides, and proteins, and C₂-C₆ alkenyl, C₂-C₆ alkynyl, aryl, arylcarbonyl, and heteroaryl, which may be optionally substituted with a substituent selected from the group consisting of hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, and guanidine;

R⁵ is H or C₁-C₆ alkoxy;

R⁹ and R¹⁰ are ethyl;

R⁶ and R⁸ are halo or hydrogen; and

X¹, X², and X³ are independently O or S;

(b) measuring the fluorescence of a fluorescent product formed during the contacting; and

(c) correlating the measured fluorescence with the activity of the organophosphatase enzyme.

20. The method of claim 19, wherein the organophosphatase is paraoxonase.

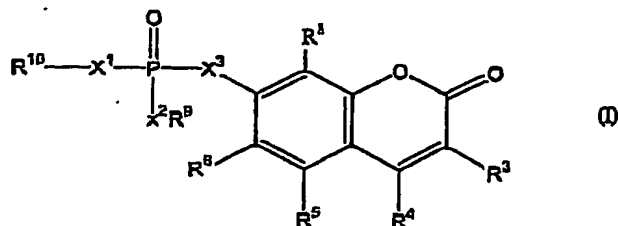
21. The method of claim 19, wherein the organophosphatase is OPH.

22. The method of claim 19, wherein R⁹ and R¹⁰ are ethyl, R⁴ is methyl, R⁶ and R⁸ are fluoro, and X¹, X², and X³ are O.

23. The method of claim 19, wherein X¹ and X² are O, X³ is S, R⁶ and R⁸ are H; R⁹ and R¹⁰ are ethyl, and R⁴ is methyl.

24. A method for specifically and selectively detecting and/or measuring the activity of an organophosphatase enzyme immobilized on a support, which comprises at least organophosphatases and phosphatases, said method comprising:

(a) contacting the support with a compound of the formula I:



wherein

R^3 is selected from the group consisting of H, cyano, C_1 - C_6 alkyl, C_1 - C_6 perfluoroalkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, aryl, and heteroaryl, formyl, carboxamide of the formula $-(C=O)NR^1R^2$ where R^1 and R^2 are independently H, alkyl having 1-6 carbon atoms, an aryl, or R^1 and R^2 taken together form a saturated 5- or 6- membered ring having the formula $-(CH_2)_2-M-(CH_2)_2-$ where the ring moiety M is a single bond, an oxygen atom, a methylene group, or the secondary amine $-NR^7-$ where R^7 is H or alkyl having 1-6 carbon atoms;

R^4 is selected from the group consisting of H, hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, sulfomethyl, salt of sulfomethyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, guanidino, C_1 - C_6 alkylamino, C_1 - C_6 acylamino, C_1 - C_6 alkylamido, C_1 - C_6 alkyl, C_1 - C_6 alkoxy, C_1 - C_6 perfluoroalkyl, halomethyl, C_1 - C_6 alkylthio, C_5 - C_8 cycloalkyl, C_1 - C_6 haloalkyl, C_5 - C_8 halocycloalkyl, C_1 - C_6 hydroxyalkyl, C_5 - C_8 hydroxycycloalkyl, C_1 - C_6 alkoxy C_1 - C_6 alkyl, C_2 - C_6 alkoxy carbonyl, C_2 - C_6 alkoxy carbonyl C_1 - C_6 alkyl, carboxy C_1 - C_6 alkyl, carboxy C_1 - C_6 alkoxy, dicarboxy C_1 - C_6 alkyl, dicarboxy C_1 - C_6 alkoxy, C_2 - C_6 cyanoalkyl, phosphono C_1 - C_6 alkyl, phosphoryl C_1 - C_6 alkyl, mono-, di-, and trisaccharides, nucleic acids, oligonucleotides, amino acids, peptides, and proteins, and C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, aryl, arylcarbonyl, and heteroaryl, which may be optionally substituted with a substituent selected from the group consisting of hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, and guanidine;

R^5 is H or C_1 - C_6 alkoxy;

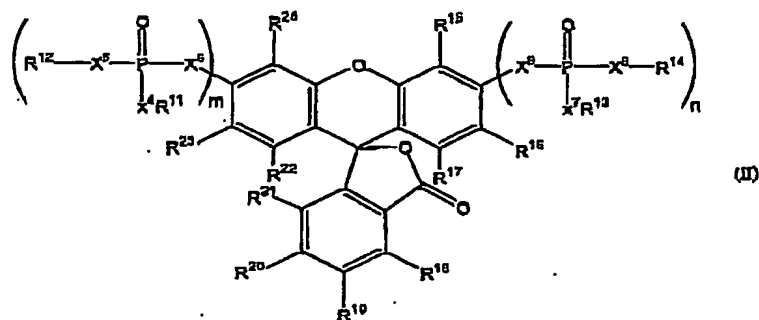
R^9 and R^{10} are ethyl;

R^6 and R^8 are halo or hydrogen; and

X^1 , X^2 , and X^3 are independently O or S;

(b) measuring the fluorescence of a fluorescent product formed during the contacting; and

- (c) correlating the measured fluorescence with the activity of the organophosphatase enzyme.
25. The method of claim 24, wherein the organophosphatase is paraoxonase.
26. The method of claim 24, wherein the organophosphatase is OPH.
27. The method of claim 24, wherein the support is a membrane, resin, biosensor, microtiter plate, nanotube or dipstick.
28. The method of claim 24, wherein R^9 and R^{10} are ethyl, R^4 is methyl, R^6 and R^8 are fluoro, and X^1 , X^2 , and X^3 are O.
29. The method of claim 24, wherein X^1 and X^2 are O, X^3 is S, R^6 and R^8 are H; R^9 and R^{10} are ethyl, and R^4 is methyl.
30. A compound of the formula II:



wherein

R^{11} - R^{14} are selected from the group consisting of C_1 - C_6 alkyl, C_5 - C_8 cycloalkyl, C_1 - C_6 haloalkyl, C_1 - C_6 perfluoroalkyl, C_2 - C_6 alkenyl, and C_2 - C_6 alkynyl, and aryl, arylcarbonyl, and heteroaryl, which may be optionally substituted with a substituent selected from the group consisting of hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, thiocyanato, aldehyde, keto, carbamoyl, urethane, ureido, and guanidino;

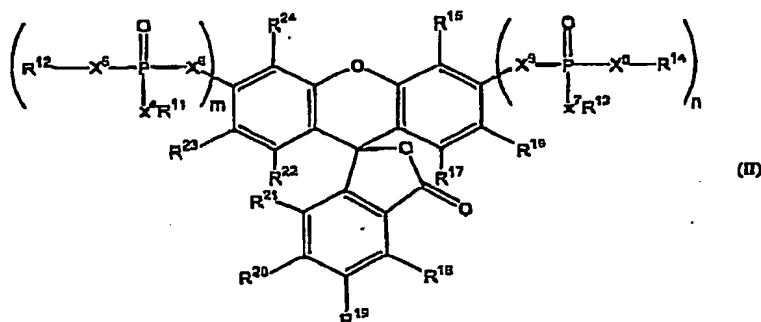
X^4 - X^9 are independently O or S;

n and m are 0 or 1 but m and n cannot be 0 simultaneously; and

R^{15} - R^{24} can be H or any substituent so long as the compound of formula II upon hydrolysis provides a fluorescent compound.

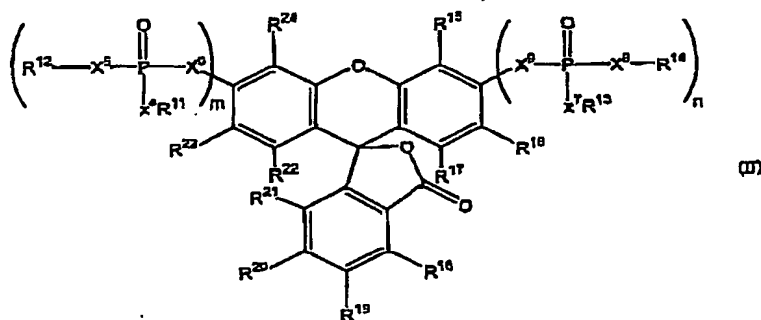
31. The compound of claim 30, wherein the hydrolysis takes place at the P-X⁶ and/or P-X⁹ bonds.
32. The compound of claim 30, wherein m and n are 1.
33. The compound of claim 30, wherein R¹⁵-R^{2a} are independently selected from the group consisting of H, hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, sulfomethyl, a salt of sulfomethyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, guanidino, C₁-C₆ alkylamino, C₁-C₆ acylamino, C₁-C₆ alkylamido, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ alkylthio, C₅-C₈ cycloalkyl, C₁-C₆ haloalkyl, C₁-C₆ perfluoroalkyl, formyl, carboxamide of the formula -(C=O)NR¹R² where R¹ and R² are independently H, alkyl having 1-6 carbon atoms, an aryl, or R¹ and R² taken together form a saturated 5- or 6- membered ring having the formula -(CH₂)₂-M-(CH₂)₂- where the ring moiety M is a single bond, an oxygen atom, a methylene group, or the secondary amine -NR⁷- where R⁷ is H or alkyl having 1-6 carbon atoms, an aryl, or R¹ and R² taken together form a saturated 5- or 6- membered ring having the formula -(CH₂)₂-M-(CH₂)₂- where the ring moiety M is a single bond, an oxygen atom, a methylene group, or the secondary amine -NR⁷- where R⁷ is H or alkyl having 1-6 carbon atoms, C₅-C₈ halocycloalkyl, C₁-C₆ hydroxyalkyl, C₅-C₈ hydroxycycloalkyl, C₁-C₆ alkoxy C₁-C₆ alkyl, C₂-C₆ alkoxycarbonyl, C₂-C₆ alkoxycarbonyl C₁-C₆ alkyl, carboxy C₁-C₆ alkyl, carboxy C₁-C₆ alkoxy, dicarboxy C₁-C₆ alkyl, dicarboxy C₁-C₆ alkoxy, C₂-C₆ cyanoalkyl, phosphono C₁-C₆ alkyl, phosphoryl C₁-C₆ alkyl, mono-, di-, and trisaccharides, nucleic acids, oligonucleotides, amino acids, peptides, and proteins, and C₂-C₆ alkenyl, C₂-C₆ alkynyl, aryl, arylcarbonyl, and heteroaryl, which may be optionally substituted with a substituent selected from the group consisting of hydroxyl, cyano, nitro, halo, amino, amido, azido, acetal, ketal, imido, sulfo, sulfonyl, sulfinyl, thiocyanato, aldehydo, keto, carbamoyl, urethane, ureido, and guanidino.
34. The compound of claim 30, wherein R¹¹-R¹⁴ are independently selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, aryl, and heteroaryl.
35. The compound of claim 30, wherein R¹¹-R¹⁴ are independently selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, and C₂-C₆ alkynyl.
36. The compound of claim 30, wherein R¹¹-R¹⁴ groups are independently selected from the group consisting of C₁-C₆ alkyl.
37. The compound of claim 30, wherein R¹¹-R¹⁴ is ethyl.

38. A compound of formula II



wherein X^4 - X^9 are O, R^{15} - R^{24} are H, R^{11} - R^{14} are ethyl; and m and n are 1.

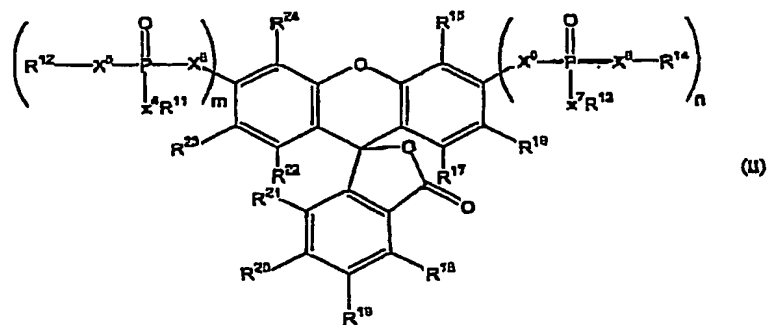
39. A compound of formula II:



wherein X^4 , X^5 , X^7 , and X^8 are O; X^6 and X^9 are S; R^{15} - R^{24} are H; R^{11} - R^{14} are ethyl; and m and n are 1.

40. A method for specifically and selectively detecting and/or measuring the activity of an organophosphatase enzyme in a fluid, which contains at least organophosphatases and phosphatases, said method comprising:

(a) contacting the fluid with a compound of the formula II:

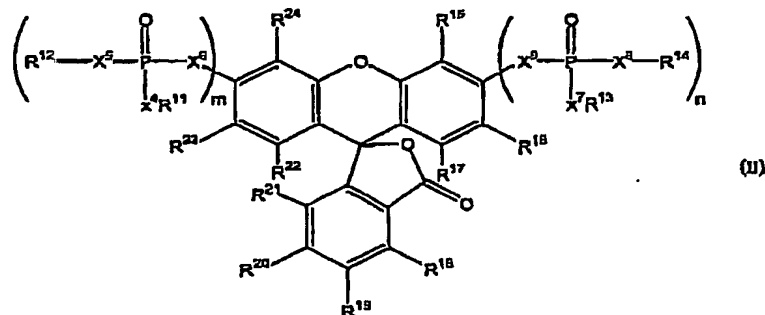


wherein R^{11} - R^{14} are selected from the group consisting of H and groups or atoms other than H, X^4 - X^9 are independently O or S, n and m are 0 or 1 but m and n cannot be 0 simultaneously, and R^{15} - R^{24} can be H or any substituent so long as the compound of formula II upon hydrolysis provides a fluorescent product;

- (b) collecting the fluorescent product;
- (c) measuring the fluorescence of a fluorescent product formed during the contacting; and
- (d) correlating the measured fluorescence with the activity of the organophosphatase enzyme.

41. A method for selectively detecting an organophosphatase enzyme in a sample suspected to contain an organophosphatase and a phosphatase comprising

- (a) contacting the sample with a compound of the formula II:

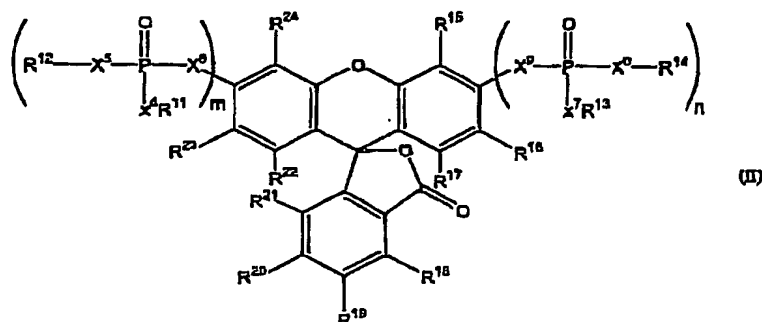


wherein R^{11} - R^{14} are selected from the group consisting of H and groups or atoms other than H, X^4 - X^9 are independently O or S, n and m are 0 or 1 but m and n cannot be 0 simultaneously, and R^{15} - R^{24} can be H or any substituent so long as the compound of formula II upon hydrolysis provides a fluorescent product;

- (b) collecting the fluorescent product;
- (c) measuring the fluorescence of a fluorescent product formed during the contacting; and
- (d) correlating the measured fluorescence with the activity of the organophosphatase enzyme.

42. A method for specifically and selectively detecting and/or measuring the activity of an organophosphatase enzyme immobilized on a support comprising:

(a) contacting the support with a compound of the formula II:



wherein R¹¹-R¹⁴ are selected from the group consisting of H and groups or atoms other than H, X⁴-X⁹ are independently O or S, n and m are 0 or 1 but m and n cannot be 0 simultaneously, and R¹⁵-R²⁴ can be H or any substituent so long as the compound of formula II upon provides a fluorescent product;

(b) collecting the fluorescent product;

(c) measuring the fluorescence of a fluorescent product formed during the contacting; and

(d) correlating the measured fluorescence with the activity of the organophosphatase enzyme.